

*Ins CB7*

WHAT IS CLAIMED IS :

- C* *5b* *91*
- 05 1. A protein possessing a specific urate oxidase activity of at least 16 U/mg and having the following sequence : (SEQUENCE ID NO.1)
- Val His Lys Asp Glu Lys Thr Gly Val Gln Thr Val Tyr Glu Met Thr Val  
Cys Val Leu Leu Glu Gly Glu Ile Glu Thr Ser Tyr Thr Lys Ala Asp Asn  
Ser Val Ile Val Ala Thr Asp Ser Ile Lys Asn Thr Ile Tyr Ile Thr Ala  
Lys Gln Asn Pro Val Thr Pro Pro Glu Leu Phe Gly Ser Ile Leu Gly Thr  
10 His Phe Ile Glu Lys Tyr Asn His Ile His Ala Ala His Val Asn Ile Val  
Cys His Arg Trp Thr Arg Met Asp Ile Asp Gly Lys Pro His Pro His Ser  
Phe Ile Arg Asp Ser Glu Glu Lys Arg Asn Val Gln Val Asp Val Val Glu  
Gly Lys Gly Ile Asp Ile Lys Ser Ser Leu Ser Gly Leu Thr Val Leu Lys  
Ser Thr Asn Ser Gln Phe Trp Gly Phe Leu Arg Asp Glu Tyr Thr Thr Leu  
15 Lys Glu Thr Trp Asp Arg Ile Leu Ser Thr Asp Val Asp Ala Thr Trp Gln  
Trp Lys Asn Phe Ser Gly Leu Gln Glu Val Arg Ser His Val Pro Lys Phe  
Asp Ala Thr Trp Ala Thr Ala Arg Glu Val Thr Leu Lys Thr Phe Ala Glu  
Asp Asn Ser Ala Ser Val Gln Ala Thr Met Tyr Lys Met Ala Glu Gln Ile  
Leu Ala Arg Gln Gln Leu Ile Glu Thr Val Glu Tyr Ser Leu Pro Asn Lys  
20 His Tyr Phe Glu Ile Asp Leu Ser Trp His Lys Gly Leu Gln Asn Thr Gly  
Lys Asn Ala Glu Val Phe Ala Pro Gln Ser Asp Pro Asn Gly Leu Ile Lys  
Cys Thr Val Gly Arg Ser Ser Leu Lys Ser Lys Leu  
preceded if appropriate, by a methionine, or having a substantial  
degree of homology with that sequence.
- 25 2. A protein according to claim 1, possessing a specific  
urate oxidase activity of at least 30 U/mg.
- Ins E1* *a*
- 30 3. A protein according to claim 1 or 2, which presents, by  
analysis on a bidimensional gel, a spot of molecular mass of about  
33.5 kDa and an isoelectric point around 8.0, representing at least  
90 % of the protein mass.
- Sub C1*
- 35 4. A protein according to anyone of claims 1 to 3, having a  
purity degree, determined by liquid chromatography on a C8 grafted  
silica column, higher than 80 %.
5. A protein according to anyone of claims 1 to 4, having an  
isoelectric point around 8.0.

6. A protein according to anyone of claims 1 to 4, which carries a blocking group on the amino-terminal serine having preferably a molecular mass around 43 units of atomic mass.

7. A drug containing a protein according to anyone of claims 1 to 6.

8. A recombinant gene which has the DNA sequence coding for the protein having the following sequence: (SEQUENCE ID NO. 2)

Met Ser Ala Val Lys Ala Ala Arg Tyr Gly Lys Asp Asn Val Arg Val  
Tyr Lys Val His Lys Asp Glu Lys Thr Gly Val Gln Thr Val Tyr Glu  
Met Thr Val Cys Val Leu Leu Glu Gly Glu Ile Glu Thr Ser Tyr Thr Lys  
Ala Asp Asn Ser Val Ile Val Ala Thr Asp Ser Ile Lys Asn Thr Ile Tyr  
Ile Thr Ala Lys Gln Asn Pro Val Thr Pro Pro Glu Leu Phe Gly Ser Ile  
Leu Gly Thr His Phe Ile Glu Lys Tyr Asn His Ile His Ala Ala His Val  
Asn Ile Val Cys His Arg Trp Thr Arg Met Asp Ile Asp Gly Lys Pro  
His Pro His Ser Phe Ile Arg Asp Ser Glu Glu Lys Arg Asn Val Gln  
Val Asp Val Val Glu Gly Lys Gly Ile Asp Ile Lys Ser Ser Leu Ser Gly  
Leu Thr Val Leu Lys Ser Thr Asn Ser Gln Phe Trp Gly Phe Leu Arg Asp  
Glu Tyr Thr Thr Leu Lys Glu Thr Trp Asp Arg Ile Leu Ser Thr Asp Val  
Asp Ala Thr Trp Gln Trp Lys Asn Phe Ser Gly Leu Gln Glu Val Arg Ser  
His Val Pro Lys Phe Asp Ala Thr Trp Ala Thr Ala Arg Glu Val Thr Leu  
Lys Thr Phe Ala Glu Asp Asn Ser Ala Ser Val Gln Ala Thr Met Tyr Lys  
Met Ala Glu Gln Ile Leu Ala Arg Gln Gln Leu Ile Glu Thr Val Glu Tyr  
Ser Leu Pro Asn Lys His Tyr Phe Glu Ile Asp Leu Ser Trp His Lys Gly  
Leu Gln Asn Thr Gly Lys Asn Ala Glu Val Phe Ala Pro Gln Ser Asp Pro  
Asn Gly Leu Ile Lys Cys Thr Val Gly Arg Ser Ser Leu Lys Ser Lys Leu

9. A recombinant gene according to claim 8, which permits the expression in the prokaryotic microorganisms.

10. A recombinant gene according to claim 9, wherein the DNA sequence contains the followings sequence: (SEQUENCE ID NO. 3):

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ATGTCTGCGG TAAAAGCAGC GCGCTACGGC AAGGACAATG TTCGCGTCTA  
CAAGGTTTAC AAGGACGAGA AGACCGGTGT CCAGACGGTG TACGAGATGA  
CCGTCTGTGT GCTTCTGGAG GGTGAGATTG AGACCTCTTA CACCAAGGCC  
GACAACAGCG TCATTGTCGC AACCGACTCC ATTAAGAACA CCATTTACAT  
CACCGCCAAG CAAACCCCG TTAATCCTCC CGAGCTGTTC GGCTCCATCC  
TGGGCACACA CTTCAATTGAG AAGTACAACC ACATCCATGC CGCTCACGTC  
AACATTGTCT GCCACCGCTG GACCCGGATG GACATTGACG GCAAGCCACA  
CCCTCACTCC TTCATCCGCG ACAGCGAGGA GAAGCGGAAT GTGCAGGTGG  
ACGTGGTCTGA GGGCAAGGGC ATCGATATCA AGTCGTCTCT GTCCGGCCTG  
ACCGTGCTGA AGAGCACCAA CTCGAGTTC TGGGGCTTCC TCGTGACGA  
GTACACCACA CTTAAGGAGA CCTGGGACCG TATCCTGAGC ACCGACGTCG  
ATGCCACTTG GCAGTGGGAG AATTTCACTG GACTCCAGGA GGTCCGCTCG  
CACGTGCCTA AGTTCGATGC TACCTGGGCG ACTGCTCGCG AGGTCACTCT  
GAAGACTTTT GCTGAAGATA ACAGTCCAG CGTGCAAGGCC ACTATGTACA  
AGATGGCAGA GCAAATCCTG GCGCGCCAGC AGCTGATCGA GACTGTGCGA  
TACTCGTTGC CTAACAAGCA CTATTTGAA ATCGACTGA GCTGGCACAA  
GGGCCTCCAA AACACCGGCA AGAACGCCGA GGTCTTCGCT CCTCAGTCGG  
ACCCCAACGG TCTGATCAAG TGTACCGTCG GCCGGTCCTC TCTGAAGTCT  
AAATTG.

11. A recombinant gene according to claim 8, which permits the expression in the eukaryotic cells.

12. A recombinant gene according to claim 11, wherein the DNA sequence contains the following sequence:

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SEQUENCE ID NO. 4

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ATGTCTGCTG TTAAGGCTGC TAGATACGGT AAGGACAACG TTAGACTCTA  
CAAGCTTCAC AAGGACGAGA AGACCGGTGT CCAGACGGTG TACGAGATGA  
CCGTCTGTGT GCTTCTGGAG GGTGAGATTG AGACCTCTTA CACCAAGGCC  
GACAACAGCG TCATTGTCGC AACCGACTCC ATTAAGAACA CCATTTACAT  
CACCGCCAAG CAGAACCCCG TTAATCCTCC CGAGCTGTTT GGCTCCATCC  
TGGGCACACA CTTCATTGAG AAGTACAACC ACATCCATGC CGCTCACGTC  
AACATTGTCT GCCACCGCTG GACCCGGATG GACATTGACG GCAAGCCACA  
CCCTCACTCC TTCATCCGCG ACAGCGAGGA GAAGCGGAAT GTGCAGGTGG  
ACGTGGTCGA GGGCAAGGGC ATCGATATCA AGTCGTCTCT GTCCGGCCTG  
ACCGTGCTGA AGAGCACCAA CTCGCAGTTC TGGGGCTTCC TGCCTGACGA  
GTACACCACA CTTAAGGAGA CCTGGGACCG TATCCTGAGC ACCGACGTCG  
ATGCCACTTG GCAGTGGAAG AATTCAGTG GACTCCAGGA GGTCCGCTCG  
CACGTGCCTA AGTTCGATGC TACCTGGGCC ACTGCTCGCG AGGTCACTCT  
GAAGACTTTT GCTGAAGATA ACAGTGCCAG CGTGCAGGCC ACTATGTACA  
AGATGGCAGA GCAAATCCTG GCGCGCCAGC AGCTGATCGA GACTGTGAG  
TACTCGTTGC CTAACAAGCA CTATTTGAA ATCGACCTGA GCTGGCACAA  
GGGCCTCCAA AACACCGGCA AGAACGCCGA GGTCTTCGCT CCTCAGTCGG  
ACCCCAACGG TCTGATCAAG TGTACCGTCG GCCGTCCTC TCTGAAGTCT  
AAATTG.

13. A recombinant gene according to claim 8, which permits the expression in the animal cells.

14. A recombinant gene according to claim 13, wherein the DNA sequence contains the following sequence: (SEQUENCE ID NO. 6)

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-ATGTC CGCAGTAAAA GCAGCCCGCT ACGGCAAGGA  
CAATGTCCGC GTCTACAAGG TTCACAAGGA CGAGAAGACC GGTGTCCAGA  
CGGTGTACGA GATGACCGTC TGTGTGCTTC TGGAGGGTGA GATTGAGACC  
TCTTACACCA AGGCCGACAA CAGCGTCATT GTCGCAACCG ACTCCATTAA  
GAACACCATT TACATCACCG CCAAGCAGAA CCCC GTTACT CCTCCCAGC  
TGTTCCGGCTC CATCCTGGGC ACACACTTCA TTGAGAAGTA CAACCACATC  
CATGCCGCTC ACGTCAACAT TGTCTGCCAC CGCTGGACCC GGATGGACAT  
TGACGGCAAG CCACACCCTC ACTCCTTCAT CCGCGACAGC GAGGAGAAGC  
GGAATGTGCA GGTGGACGTG GTCGAGGGCA AGGGCATCGA TATCAAGTCG  
10 TCTCTGTCCG GCCTGACCGT GCTGAAGAGC ACCAACTCGC AGTTCTGGGG  
CTTCCTGCGT GACGAGTACA CCACACTTAA GGAGACCTGG GACCGTATCC  
TGAGCACCGA CGTCGATGCC ACTTGGCGGT GGAAGAATTT CAGTGGACTC  
CAGGAGGTCC GCTCGCACGT GCCTAAGTTC GATGCTACCT GGGCCACTGC  
TCGCGAGGTC ACTCTGAAGA CTTTGCTGA AGATAACAGT GCCAGCGTGC  
15 AGGCCACTAT GTACAAGATG SCAGAGCAAA TCCTGGCGCG CCAGCAGCTG  
ATCGAGACTG TCGAGTACTC GTTGGCTAAC AAGCACTATT TCGAAATCGA  
CCTGAGCTGG CACAAGGGCC TCCAAAACAC CGGCAAGAAC GCCGAGGTCT  
TCGCTCCTCA GTCGGACCCC AACGGTCTGA TCAAGTGATC CGTCGGCCGG  
TCCTCTCTGA AGTCTAAATT G

20 preceded by a non-translated 5' sequence favoring expression in animal cells.

15. Recombinant gene according to claim 14, wherein the non-translated 5' sequence favoring expression in animal cells comprises the sequence AGCTTGGCGCCACT, located immediately upstream from the sequence described in claim 14.

25 16. An expression vector carrying a recombinant gene according to any one of claims 8 to 15 with the means necessary for its expression.

17. An expression vector according to claim 16, which carries at least one selection marker.

30 18. An expression vector according to claim 17, which has the characteristics of one of plasmids pEMR469, pEMR473, and pEMR 515.

19. Prokaryotic microorganisms which are transformed by an expression vector according to claim 16, carrying a recombinant gene according to claim 9.

20. Eukaryotic cells which are transformed by one of the expression vectors according to any one of claims 16 to 18, carrying the recombinant gene according to claim 11.
- 05 21. A strain of Saccharomyces cerevisiae which is transformed by one of the expression vectors according to any one of claims 16 to 18.
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22. A strain according to claim 21, which carries a mutation on at least one of the genes responsible for the synthesis of leucine or uracil.
- 10 23. A strain according to claim 22, which carries a mutation on at least one of the LEU2 and URA3 genes.
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24. A process for producing a recombinant urate oxidase which comprises the steps of :
- 15 1) cultivating a strain according to claims 21 to 23 ;
- 2) lysing the cells ;
- 3) isolating and purifying the recombinant urate oxidase contained in the lysate.
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25. Animal cells containing a recombinant gene according to claim 13 with the means necessary for its expression.
- 20 26. Animal cells containing an expression vector according to claim 16, carrying a recombinant gene according to claim 14.
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*add*  
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